STUDIES IN THE NITROGEN METABOLISM OF BACTERIA

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The experiments here recorded were undertaken with the idea of gaining some information, if possible, as to the materials which usually serve as a source of nitrogen to bacteria, the actual form in which the nitrogenous substances are absorbed, and whether all simple dialyzable protein decomposition products are of equal usefulness in building up the cell substance.

The bulk of investigations of proteolysis in the past has had to do with the identification or determination of end-products. The early researches using mixed cultures on putrescible materials, although not usually controlled as to the exact chemical constituents of the medium, or the strains of bacteria employed, showed that the formation of certain substances such as ammonia, hydrogen sulphid, skatol, and so forth is characteristic of putrefaction irrespective of the precise nature of the protein or the particular varieties of organisms present. Productive of much more knowledge of actual bacterial metabolism was the long list of researches using pure cultures and mediums of known composition, making tests to determine the nature of the compounds formed. The indol test is the best known and most useful result of the investigations carried out on peptones or other tryptophane-containing medium.

Herter and Broeck[1] using a 1% preparation of casein with B. proteus demonstrated the presence of primary amins, hydrogen sulphid, fatty acids, aromatic oxyacids, indol, and indol acetic acid. Nawiasky[2] compared the products used up by B. proteus on a medium rich in peptone with those assimilated by Vibrio Finkler, B. fecalis-alkaligenes, and B. mesentericus, and states that B. proteus attacks albuminoses much more actively than do the others, but that its action on peptones and creatin was less marked. Rettger[3] planted B. putrificus Bienstock, B. edematis maligni, B. anthracis-symptomatici, B. coli, and B. lactis-aerogenes on special egg-meat medium and tested for indol, skatol, phenol, aromatic oxyacids, skatol-carbonic acid, tyrosin, leucin, albumoses, peptones, tryptophane, hydrogen sulphid, and mercaptan, his con-

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[1] Jour. Biol. Chem., 1911, 9, p. 491.
[2] Arch. f. Hyg., 1908, 64, p. 33.
[3] Jour. Biol. Chem., 1906, 2, p. 71.

clusion being that only the obligate anaerobes cause true putrefaction. Later with Newell[4] he tried 26 strains of B. proteus on egg-meat medium, blood fibrin suspended in broth, and Uschinsky's medium and concluded that, while actively proteolytic, B. proteus does not form those end-products characteristic of putrefaction in the narrow sense of the word. Effront[5] studied B. proteus, B. sporogenes, B. mesentericus, B. butyricus, B. putrificus Bienstock, etc., in a similar manner on albuminoid mediums. Sasaki's[6] researches on the particular substances formed from glycyl-glycin, glycyl tyrosin, cystin and tyrosin are of interest and value as pictures of a certain stage of protein degradation, as are the works of Brasch[7] on glutamic acid, serin, and tyrosin.

While investigations like those mentioned are of diagnostic importance, and throw much light on the types of chemical activity of which a given bacterium is capable, a definite idea of the metabolism of an organism cannot be obtained from purely qualitative data or from a single quantitative determination of some particular product. For such purposes a series of quantitative determinations should be made on a favorable medium, under conditions of vigorous normal growth. Many factors enter into the nature and amount of decomposition products formed: first, the availability of the nitrogenous compounds present, depending on whether or not the organism in question is capable of elaborating a ferment to digest them, the molecular point of attack of the enzyme, whether intermediate products are present, and, if so, what and in what quantity, what proportion of the split molecules are resynthesized into bacterial protein and which are left in the medium, and whether or not some of the compounds present are assimilated in preference to others. In the case of any particular end-product it is desirable to know whether it represents a portion of the protein molecule unattacked by the bacteria, a true cell excretion, or something set free by the death of the cell or by autolysis. Any systematic investigation of the proteolytic activity of an organism should include at least the following points:

1. Determination of the amounts present of certain available food substances.

2. Periodic determination of the amounts of one or more decomposition products used as a measure of proteolysis.

3. Allowances for such factors as rate of increase in numbers, presence of other non-nitrogenous foodstuffs, presence of decomposi-

[4] Jour. Biol. Chem., 1912, 13, p. 341.

[5] Monit. Scient. Quesneville, 1911, I, p. 489.

[6] Biochem. Ztschr., 1912, 41, p. 174; 1912, 47, pp. 462-472; 1914, 59, p. 429; Sasaki, Takaoki and Otsuka: Biochem. Ztschr., 1912, 39, p. 208.

[7] Biochem. Ztschr., 1909, 18, p. 380; 1909, 22, p. 403.

tion products toxic to the cells or inhibitory of their activity, and products of autolysis of dead cells.

The infinite complexity of proteins, their delicate susceptibility to change under the influence of chemical and physical agents used for their isolation and purification, and the purely technical difficulties involved in an attempt to avoid accidental contaminations while preserving optimum conditions of growth as well as the refinements of chemical technic necessary to quantitative determination, have tended to discourage this type of research, with the result that there are comparatively few such studies on record, all more or less incomplete.

Among the end-products of putrefaction which have been determined quantitatively as a measure of proteolysis, ammonia has been the most common. Effront[8] made use of it in his studies on yeast. Emmerling and Reiser[9] stated that at least 25% of the nitrogen in gelatin was converted into ammonia by B. fluorescens-liquefaciens, while Arnaud and Charrin[10] found that in three days B. pyocyaneus converted 91% of the nitrogen in asparagin into ammonia and that a 3 weeks' culture in gelatin contained ammonia to the amount of 70% of the total nitrogen. Boencke[11] and Kendall and Farmer[12] determined ammonia to check the degree of proteolysis in their studies on the protein-sparing influence of carbohydrate. While the information thus obtained is valuable, particularly in the case of the putrefactive saprophytes, the ammonia curve admits of varying interpretations unless checked by other determinations. Moreover, many pathogens do not liberate much ammonia during their short period of life in artificial cultures (Berghaus[13]). The quantitative determination of indol and of phenol, valuable as they are in the study of B. coli and similar organisms, has the same limitation.

At the other extreme of the metabolic process, the progress of proteolysis may be observed by systematic determinations of the amount of coagulable protein remaining in the culture medium. De Waele and Vendevelde[14] worked out these results with a variety of organisms planted on gelatin, milk, and casein broth in an endeavor to settle the question of the specificity of bacterial proteases. Bainbridge,[15] Rettger and Sperry[16] working with representative pathogenic and saprophytic strains on medium composed of mineral salts and pure protein (crystallized egg albumin, serum protein, alkali albumin, and edestin) have proven that such growth as may occur on solutions of unchanged proteins is due to the minute amounts of nitrogenous impurities present, and that in the absence of split-protein products the germs are unable to attack native albumin.

[8] Compt. rend. Acad. de sc., 1908, 146, p. 779.
[9] Ber. d. deutsch. chem. Gesellsch., 1902, Part 3, p. 700.
[10] Compt. rend. Acad. de sc., 1891, 112, p. 755.
[11] Arch. f. Hyg., 1911, 74, p. 81.
[12] Jour. Biol. Chem., 1912, 12, p. 13; 1912, 13, p. 63.
[13] Arch. f. Hyg., 1908, 64, p. 1.
[14] Centralbl. f. Bakteriol., I, O., 1903, 39, p. 353.
[15] Jour. Hyg., 1911, II, p. 341.
[16] Am. Jour. Physiol., 1902, 8, p. 284.
Sperry: Report at Soc. Am. Bact., Montreal, Dec. 31, 1913.
Sperry and Rettger: Jour. Biol. Chem., 1914, 20, p. 445.

The quantitative determination of amino-acids is particularly useful, especially if checked by comparison with the amount of destruction of the protein or split-protein which is their source, or, in experiments using the amino-acids as a source of nitrogenous food, estimations of the ammonia which is their fate (Nawiasky[17]). Until very recently Sörensen's formol-titration method[18] has been the most in use. This procedure was followed by Rosenthal and Patai[19] who planted virulent and avirulent strains of staphylococcus, streptococcus and B. coli on broth. With all three of these organisms they observed an initial sharp rise of amino-acid, followed by a more gradual rate of increase. Meserintsky[20] observed a progressive augmentation of the amount of amino-acid present in a 10% gelatin culture of B. prodigiosus, which, however, represented only a portion of the non-coagulable nitrogenous material present. Frouin and Ledebt,[21] working with B. coli, B. typhosus, B. dysenteriae and V. cholerae on a solution of non-nitrogenous mineral salts enriched with amino-acids were unable to detect any decrease during the first 24 hours of growth, though the diminution became apparent later. Kendall, Day and Walker[22] attempted to use Sörensen technic in addition to determinations of ammonia, total nitrogen and acidity in their work on the protein-sparing action of glucose, but abandoned the amino-acid estimation because "the results furnished no information of importance."

The more delicate and accurate method of Van Slyke[23] now makes it possible to obtain much more significant estimation of the progress of proteolysis, since the micro-apparatus is accurate to 0.005 milligram of nitrogen.

This method was used by Sears[24] on peptone, broth, and gelatin cultures of a great variety of organisms. He also determined ammonia and creatinin. While some of his cultures showed a gradual increase in amino-nitrogen, the majority exhibited fluctuating rises and falls indicating in his opinion that amino-acids were formed and broken continuously during the life of the culture.

To arrive at a more nearly complete understanding of the nitrogen metabolism of bacteria it would seem valuable not only to apply such a series of tests to growth on peptone-containing mediums, but also to study the amino-acid content of cultures on some material which contains native albumin. This is particularly true of the pathogens, many of which do not thrive on plain peptone or broth. Moreover, their behavior in a medium closely resembling blood-serum or some other body fluid is well worth detailed consideration, both for the insight it might give into the process of infection, and the possibility of the usefulness of such data in attacking the chemical side of problems of immunity. Ascitic fluid is more similar to blood serum than any other material which can easily be obtained sterile in sufficient quantity for such purposes. Accordingly, determinations have been made on B. typhosus, B. proteus, B. pyocyaneus, and staphylococcus grown on broth, on pure ascitic fluid, and on mixtures of equal quantities of the two. In order to rule out the protein-sparing properties of carbohydrates as much as possible in the interests of simplicity, sugar-free broth was used. Such broth may

[17] Arch. f. Hyg., 1908, 66, p. 209.
[18] Biochem. Ztschr., 1907, 7, pp. 45 and 407.
[19] Centralbl. f. Bakterial., I, O., 1914, 73, p. 406.
[20] Biochem. Ztschr., 1910, 29, p. 104.
[21] Compt. rend. Soc. de biol., 1911, 70, p. 24.
[22] Jour. Am. Chem. Soc., 1913, 35, p. 1201.
[23] Jour. Biol. Chem., 1911, 9, p. 185; 1912, 12, p. 275; 1913, 16, p. 121; 1915, 23, p. 407.
[24] Jour. Infect. Dis., 1916, 19, p. 105.

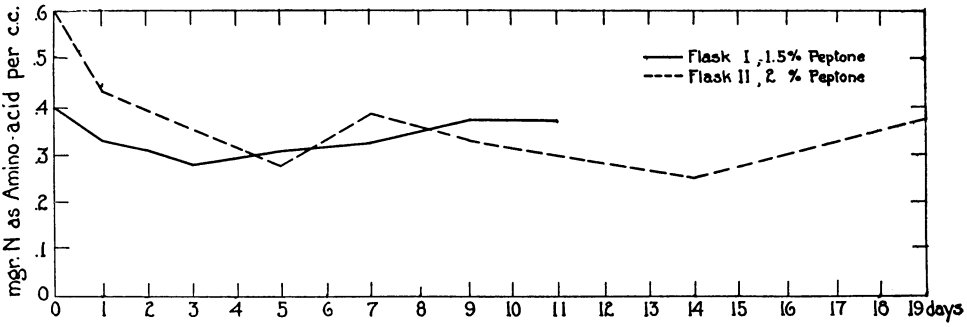
contain considerable amounts of amino-acid determined by Van Slyke's method in addition to the peptones, but no coagulable albumin or carbohydrates. In ascitic fluid, on the other hand, the amount of amino-acid and other protein decomposition products is small, while the nitrogen present as coagulable protein is considerable. A small amount of carbohydrate is probably present, but no tests for it were made. A mixture of equal parts of broth and ascitic fluid represents a middle ground between these conditions. In the experiments recorded in tables 4 and 10 ascitic fluid was distributed in equal amounts (10 c c) in a series of sterile test tubes, and each inoculated with a loopful of broth culture. At regular intervals the amino-acid content of one tube was determined, the loss by evaporation being carefully made up with distilled water. While this method has the advantage of permitting determinations to be made without danger of contamination of the material destined for later analysis, there is always the possibility of a considerable variation in the seeding of the different tubes or even in the samples of ascitic fluid. The rest of the work was done with flasks containing a quarter of a liter or more of the medium. Tight fitting rubber caps over the cotton stoppers protected against evaporation. At intervals samples were removed with sterile pipettes after shaking the flask to render the medium homogeneous. These were centrifuged and the supernatant liquid (practically, though probably not absolutely, free of bacterial cells) was tested for the amount of amino-acid present, and in some cases for coagulable protein. In cases where numbers of bacteria present are given, they were determined by counting agar plates made with the customary quantitative precautions. The Kjeldahl determinations were run in duplicate, and the results averaged if they did not check but showed a difference of less than 0.5 c c in the titration. Figures showing a wide variation were discarded. Two Van Slyke determinations were made routinely, checked by a third in case the amounts of nitrogen liberated did not agree within 0.03 c c.

TABLE 1

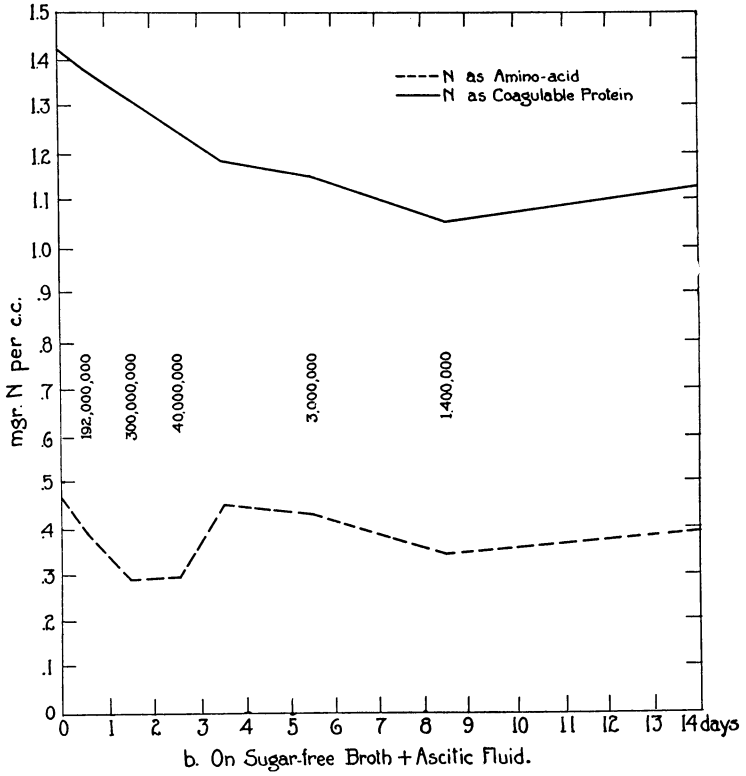
STAPHYLOCOCCUS

	Milligrams N As Amino-Acid per C C in Broth	
Incubation	Flask 1 (2% Peptone)	Flask 2 (1.5% Peptone)
Sterile	0.606	0.407
1 day	0.438	0.333
2 days	.....	0.315
3 days	.....	0.280
5 days	0.280	0.319
7 days	0.390	0.328
9 days	0.332	0.376
11 days	.....	0.369
14 days	0.252	
19 days	0.380	

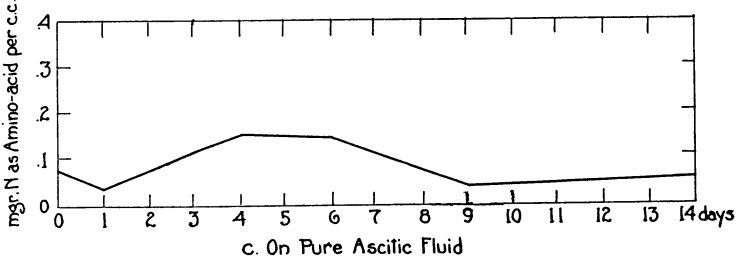
The staphylococcus cultures on broth (table 1, chart 1 a) show a drop to 0.29 mg. though flask 1 originally contained nearly half again as much amino-acid as flask 2. Flask 1 on the chart is shown as having reached this minimum two days later than flask 2 but this may well be due to the fact that no 3-day determination was recorded. In flask 2 there was a steady gradual rise after reaching the minimum until the point when it had to be discarded on account of contamination. Flask 1 shows fluctuations which are without significance when not checked by determinations of other substances.



a. On Sugar-free Broth.



b. On Sugar-free Broth + Ascitic Fluid.



c. On Pure Ascitic Fluid

CHART I. STAPHYLOCOCCUS.

On a mixture of broth and ascitic fluid (table 2 and chart 1 b) the amino-acid curve shows a decided fall during the period of most rapid multiplication, followed by a rise and later some fluctuation.

TABLE 2

STAPHYLOCOCCUS

Milligrams N per C C As Amino-Acid and As Coagulable Protein in a Mixture of Ascitic Fluid and Broth			
Incubation	Amino-Acid	Coagulable Protein	Bacteria per C C
Sterile	0.468	1.420	
12 hours	0.393	1.382	192,000,000
36 hours	0.295	1.312	300,000,000
60 hours	0.306	.....	40,000,000
84 hours	0.445	1.189	
132 hours	0.425	1.158	3,000,000
8½ days	0.341	1.052	1,400,000
14 days	0.390	1.134	

On pure ascitic fluid (table 3, chart 1 c) although the amount of amino-acid present to begin with was much smaller than in any of the other flasks, we observe the same initial drop followed by a slight rise and a subsequent fall.

TABLE 3

STAPHYLOCOCCUS

Milligrams N As Amino-Acid per C C of Pure Ascitic Fluid							
Incubation.....	Sterile	1 day	3 days	4 days	6 days	9 days	14 days
Milligrams N.....	0.067	0.033	0.112	0.145	0.143	0.033	0.054

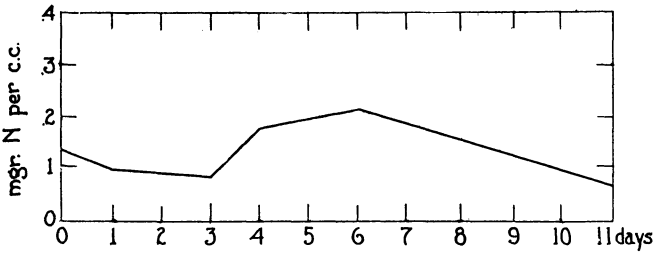
Apparently this organism readily assimilates a part at least of the amino-acids found in each of these mediums but attacks the coagulable protein to some extent from the first. After about 3 days there comes a period when amino-acids accumulate, apparently due partly to autolysis and partly to the fact that the original content of assimilable amino-acid is exhausted, and only a portion of those liberated from the proteins, peptones, etc., can be used by the organism. The increase of coagulable protein after the 8th day may be due to the fact that, as the cultures grew older, it became increasingly difficult to get a clear supernatant fluid by centrifugation.

The results obtained in similar mediums with B. pyocyaneus are noticeably different. On pure ascitic fluid (table 4, chart 2 c), after an initial drop, a steady rapid rise in amino-acid is observed.

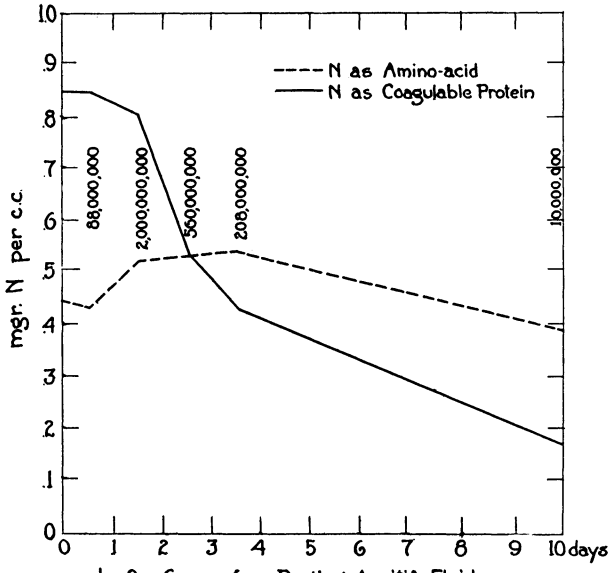
TABLE 4

B. PYOCYANEUS

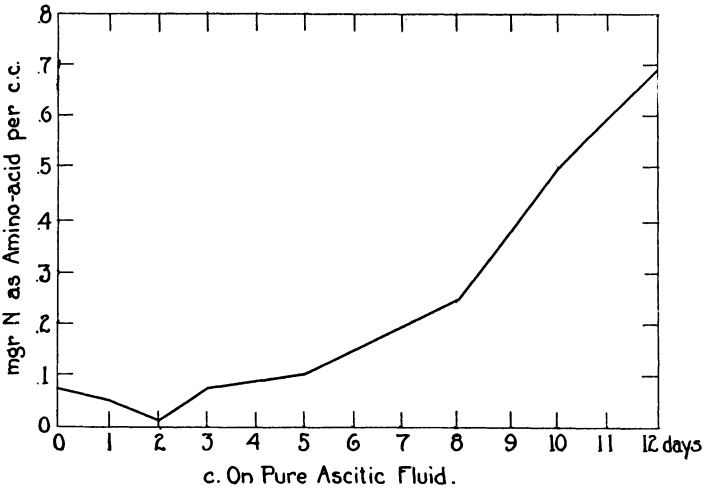
Milligrams N As Amino-Acid per C C of Ascitic Flud								
Incubation.....	Sterile	1 day	2 days	3 days	5 days	8 days	10 days	12 days
Milligrams N.....	0.077	0.049	0.013	0.074	0.099	0.249	0.493	0.695



a. On Sugar-free Broth.



b. On Sugar-free Broth + Ascitic Fluid.



c. On Pure Ascitic Fluid.

Chart II. Bacillus Pyocyaneus.

On sugar-free broth, however, there is a variation (table 5, chart 2 a) which may be significant. After the original drop (which would perhaps be more noticeable if a 2-day determination had been made) and a subsequent increase between the 3rd and 6th days, there is an unmistakable drop shown on the 11th day. Apparently, after exhausting the favorite amino-acids present in the peptone, the organism attacks the previously unused amino-acid while in an abundance of available protein it continues uneconomically to select for synthesis the molecules best adapted to its needs.

TABLE 5

B. PYOCYANEUS

	Milligrams N As Amino-Acid per C C of Broth					
Incubation.....	Sterile	1 day	3 days	4 days	6 days	11 days
Milligrams N.....	0.1384	0.098	0.086	0.184	0.215	0.066

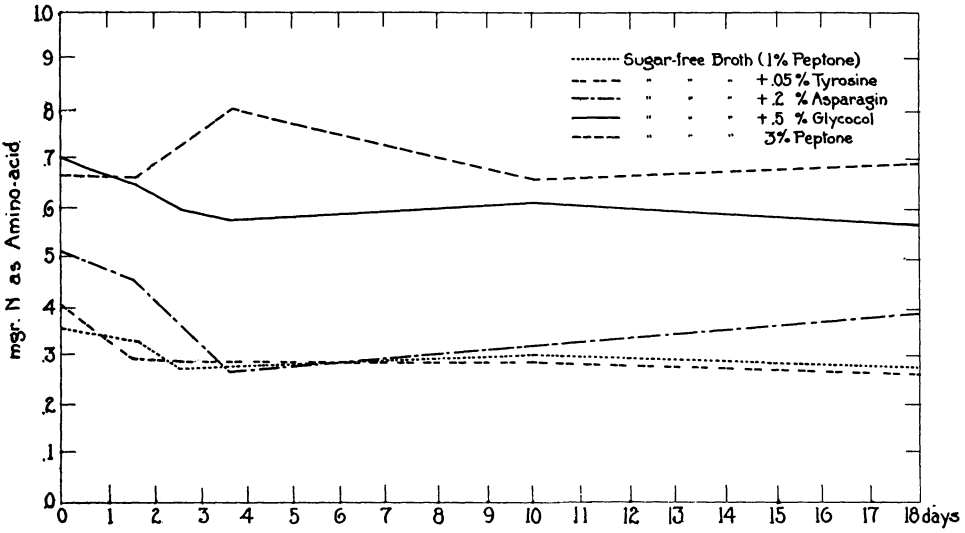
On a mixture of broth and ascitic fluid (table 6, chart 2 b) the results are somewhat similar, except that the rise in amino-acid occurred earlier. Here, too, we have a decrease in amino-nitrogen after the fourth day, although there is destruction of protein going on at the same time. Curiously enough, the greatest destruction of protein took place after the maximum count had been passed and at a time when the free amino-acids were not noticeably increasing. The protein destruction might be attributed to the activity of the enzymes previously secreted by the bacteria but in that case an increase of amino-acid would be expected. The fact that the acids do not remain free in the culture can be explained on either of two suppositions: that the enzymes secreted were of more than one kind and could carry proteolysis beyond the amino-acid stage or, on the other hand, that cell division and consequently synthesis of amino-acid into bacterial protoplasm was proceeding at a maximum rate during this period, the apparent decrease in numbers being due to the fact that the death rate began to make itself felt at the point when the count was 2,000,000,000.

TABLE 6

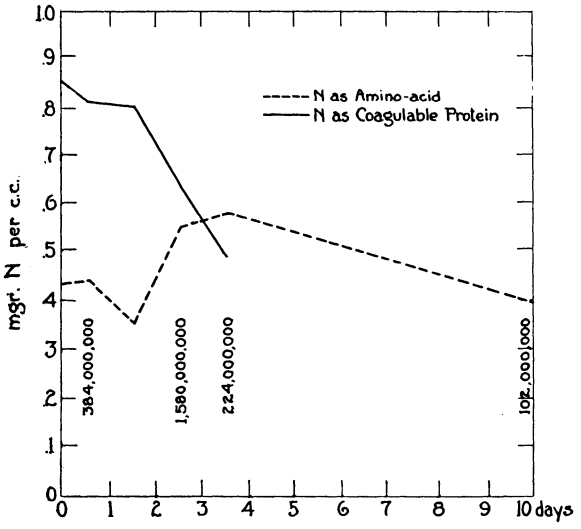
B. PYOCYANEUS

	Milligrams N per C C As Amino-Acid and As Coagulable Protein on Ascitic Fluid + Sugar-Free Broth		
Incubation	Amino-Acid	Coagulable Protein	Bacteria per C C
Sterile	0.441	0.846	
12 hours	0.439	0.846	88,000,000
36 hours	0.520	0.818	2,000,000,000
60 hours	.....	0.529	560,000,000
84 hours	0.537	0.433	208,000,000
10 days	0.386	0.172	10,000,000

In order to throw more light on this apparent preference for some protein-decomposition products instead of others, it seemed useful to investigate the effects of the addition of varying amounts and kinds of amino-acids to culture mediums. Accordingly, 0.1 c c of 48-hour broth culture of B. typhosus was inoculated into each of five flasks of sugar-free broth which differed only in that flask 1 was ordinary sugar-free broth containing 1% peptone, flask 2 contained in addition 0.05% tyrosin, flask 3 0.2% asparagin, flask 4 0.5% glycocol, and flask 5 extra peptone making a strength of 3%. The results are given in table 7 and represented graphically in chart 3 a.



a. On Sugar-free Broths of different Amino-acid Content.



b. On Sugar-free Broth + Ascitic Fluid.

CHART III. BACILLUS TYPHOSUS.

TABLE 7

B. TYPHOSUS

Milligrams N As Amino-Acid per C C on Broths of Varying Amino-Acid Content					
Incubation	1% Peptone	0.05% Tyrosin 1% Peptone	0.2% Asparagin 1% Peptone	0.5% Glycocol 1% Peptone	3% Peptone
Sterile	0.357	0.401	0.520	0.715	0.678
16 hours	.....	0.343			
18 hours	.....	.....	.....	0.686	
36 hours	0.321	0.295			
38 hours	.....	.....	0.452	0.651	0.667
60 hours	0.277	0.282	.....	0.605	
90 hours	.....	.....	0.272	0.577	0.806
10 days	0.309	0.315	.....	0.619	0.663
18 days	0.277	0.265	0.389	0.567	0.686

In the flask of plain sugar-free broth we note a gradual decrease in amino-acid during the first two days. This period covers the lag-phase and the period of most rapid multiplication, during which it uses those acids present in the medium which the organism is especially able to assimilate. The fact that no great change was apparent at the times when subsequent tests were made would seem to indicate that the peptone in the culture was being used, and that the resulting decomposition products were either synthesized into bacterial protoplasm or else carried beyond the amino-acid stage. However, there seems to be a certain amount of amino-acid present which remains untouched, doubtless being unsuitable as nutriment for the cells. In the tyrosin flask the same level is reached and maintained after 60 hours, indicating that the extra foodstuff in that form was readily assimilated. The flask with asparagin shows the same process in a more striking degree. It is unfortunate that no 60 hour test was made to determine whether the asparagin was used up in that length of time, or really required 3½ days to reach the dead level as the chart seems to show. The flask which contained a considerable amount of glycocol shows a similar absolute decrease, but the level maintained is higher than that of the other flasks by just about the amount of glycocol added. (The difference in amino-acid nitrogen between the plain broth flask and the one with glycocol was 0.358 mg. per c c when sterile, and 0.354 mg. after 10 days' incubation.) This apparently indicates that glycocol is not assimilated by B. typhosus when other sources of nitrogen are present. In the 3% peptone flask there is very little decrease during the first 1½ days, indicating that the free amino-acid used up is balanced by the fact that some is being set free from the peptone. After this, apparently the peptone destruction sets free more acid than is used up, but later still these are synthesized or more likely broken down into simpler substances. (Sears found that B. typhosus formed considerable amounts of ammonia on peptone solution.)

These results are confirmed and explained by an experiment using a medium consisting of mineral salts, glycerol and a pure amino-acid. Absolutely no visible growth of B. typhosus could be determined in such a medium with glycocol as a sole source of nitrogen, although it grew well on this medium when other amino-acids were introduced, and several other bacteria produced a heavy growth on the glycocol. In the typhoid-glycocol culture tubes there was no appreciable diminution of the amount of amino-acid present during 3 weeks, though the bacilli introduced remained viable throughout the period of observation.

The results obtained with the same strain on a mixture of sugar-free broth and ascitic fluid (table 8, chart 3 b) are of interest in connection with the first experiment.

TABLE 8

B. TYPHOSUS

Milligrams N per C C As Amino-Acid and As Coagulable Protein in a Mixture of Broth and Ascitic Fluid			
Incubation	Amino-Acid	Coagulable Protein	Bacteria per C C
Sterile	0.430	0.859	
12 hours	0.439	0.818	384,000,000
36 hours	0.354	0.805	
60 hours	0.551	0.639	1,580,000,000
84 hours	0.581	0.488	224,000,000
10 days	0.386	Lost	102,000,000

Here the protein is attacked from the first, and there is even a slight rise in amino-acid during the first 12 hours, then a considerable fall in the next 24, followed by a rapid rise in free amino-acid, and great destruction of protein continuing even a little after the maximum count was reached. Then ensues a period during which the amino-acids are gradually used up by the germs.

The two flasks of ordinary sugar-free broth inoculated with B. proteus (table 9, chart 4 a) indicate that, after the first drop due to the utilization of the amino-acids present, very little of the amino-acid broken from the peptone is left free in the medium, and the accumulation is not great.

TABLE 9

B. PROTEUS

Milligrams N As Amino-Acid per C C of Broth		
Incubation	Flask 1	Flask 2
Sterile	0.329	0.329
1 day	0.239	0.233
2 days	0.191	0.202
6 days	0.230	0.235
8 days	0.282	0.282

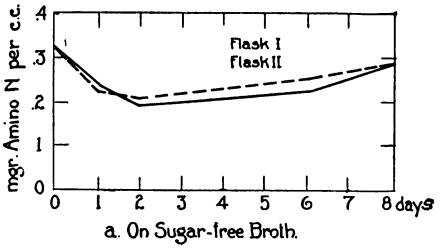
On undiluted ascitic fluid (table 10, chart 4 c) this is even more marked. Unfortunately, determinations of coagulable protein were not made, but the idea that the small amount of free amino-acid is due to thoroughness of decomposition rather than to lack of protein is strengthened by the fact that this was the same sample of fluid on which B. pyocyaneus set free 0.695 mg. per c c in 12 days.

TABLE 10

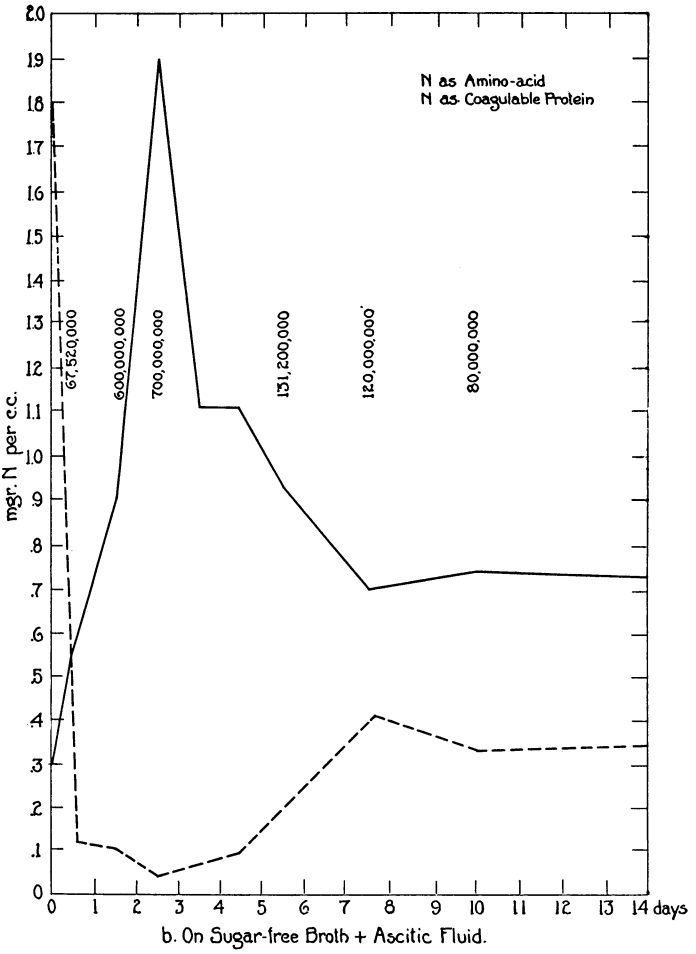
B. PROTEUS

Milligrams N As Amino-Acid per C C of Pure Ascitic Fluid									
Incubation	Sterile	1 day	2 days	3 days	4 days	5 days	6 days	7 days	12 days
Milligrams N	0.077	0.060	0.054	0.043	0.026	0.031	0.054	0.032	0.026

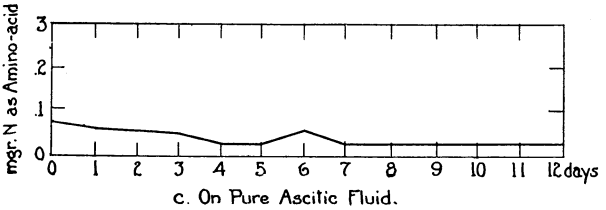
On the mixture of broth and ascitic fluid (table 11, chart 4 b) the striking destruction of coagulable protein, and the very large amount of amino-acid liberated in the first 2½ days apparently indicates that this organism attacks both protein and peptones whenever they are present, indiscriminately, and uneconomically in the case of a medium rich in both. Later much of this surplus amino-acid was used up.



a. On Sugar-free Broth.



b. On Sugar-free Broth + Ascitic Fluid.



c. On Pure Ascitic Fluid.

CHART IV. BACILLUS PROTEUS.

TABLE 11

B. PROTEUS

Incubation	Sugar-Free Broth Plus Ascitic Fluid: N per C C As NH_2	N per C C Coagulable Protein	Count
Sterile	0.307	1.807	0
14 hours	0.575	0.119	67,520,000
36 hours	0.900	0.105	600,000,000
2½ days	1.980	0.049	700,000,000
3½ days	1.175		
4½ days	1.173	0.091	
5½ days	0.934	....	131,200,000
7½ days	0.707	0.412	120,000,000
10 days	0.743	0.337	80,000,000
2 weeks	0.732	0.348	

In this flask as in the staphylococcus flask already mentioned, it was almost impossible to obtain a clear fluid by centrifugation after the 1st week, and this probably accounts for the rise in coagulable protein noted on the 7th day.

It is interesting to compare the results on the flasks of broth reinforced with various amino-acids (table 12, chart 5) with the results on plain broth as well as with the behavior of B. typhosus.

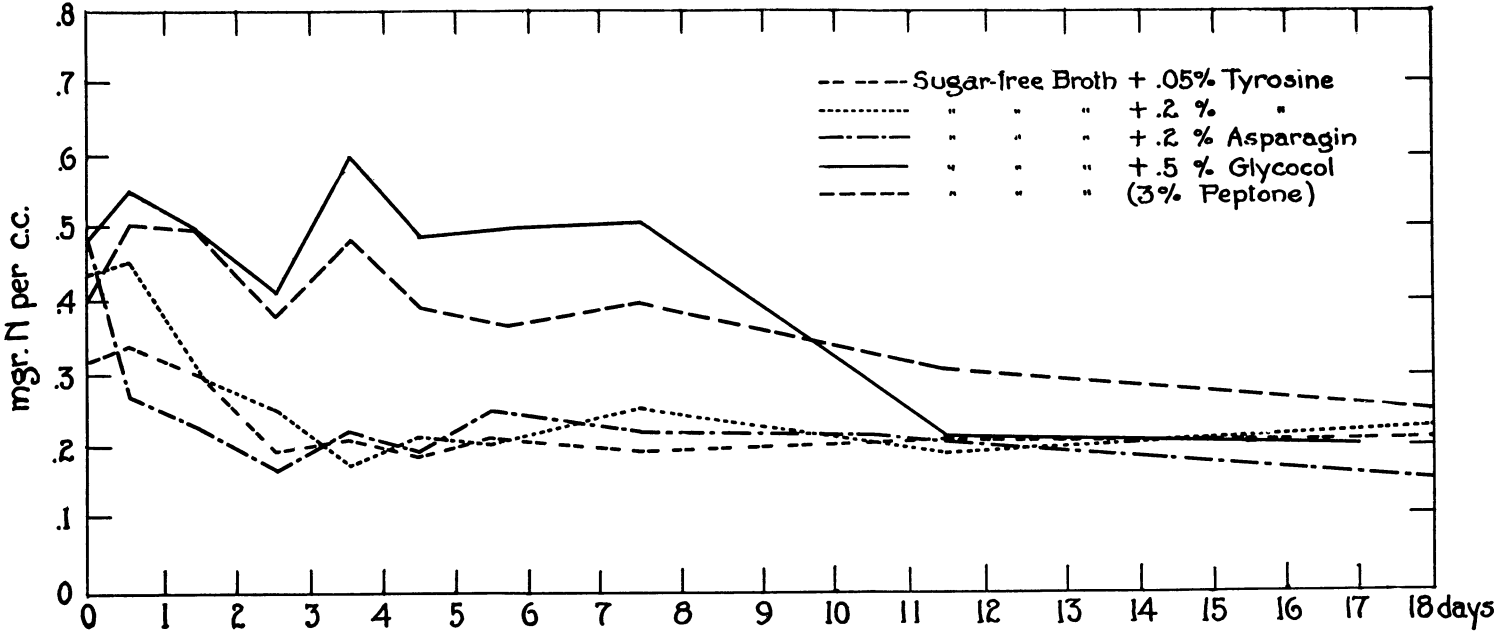
TABLE 12

B. PROTEUS

	0.05% Tyrosin	0.2% Tyrosin	0.2% Asparagin	0.5% Glycocol	3% Peptone
Sterile	0.327	0.440	0.469	0.480	0.402
12 hours	0.345	0.449	0.276	0.556	0.503
36 hours	0.309	0.292	0.220	0.496	0.499
60 hours	0.199	0.254	0.165	0.417	0.386
84 hours	0.209	0.175	0.219	0.602	0.481
4½ days	0.188	0.210	0.190	0.485	0.397
5½ days	0.212	0.201	0.257	0.491	0.372
7½ days	0.199	0.255	0.221	0.518	0.408
10½ days	.....	.....	0.219		
11½ days	0.209	0.188	.....	0.208	0.297
17 days	.....	. ...	.....	0.204	
18 days	0.209	0.222	0.155	.....	0.255

Here only one flask showed an initial fall in amino nitrogen content, namely, that containing asparagin. This substance is apparently assimilated with considerable vigor at the same time that the peptone is being broken down, and the "dead level" of useless amino-acid is reached in 2½ days. In the case of tyrosin the amounts set free are in excess of the amounts liberated during the first twelve hours but in a few days the content is about the same as that of the asparagin flask. The glycocol flask shows a very different curve from that produced with B. typhosus. Here we have an initial rise due to the attack on peptone, followed by fluctuations and a 3-day period of no great change, and then a rapid fall between the 7th and 11th days, when the "dead level" was reached. In the 3% peptone flask the initial increase is more marked than in any of the others, which is what might be expected, considering the readiness of the organism to attack such substances, and the fact that there is three times as much peptone present as in each of the other four flasks.

The results on the synthetic medium (mineral salts, glycerol and a single amino-acid) agree with these findings. While B. proteus did not use up



On Sugar-free Broths of different Animo-acid Content.

CHART V. BACILLUS PROTEUS.

glycocol as rapidly as some other bacteria did, it grew fairly well, proving that it can derive nitrogen from this acid if "starved to it." These peculiarities seem worthy of further study. It was thought possible that this difference might have some bearing on the liquefaction of gelatin, which yields a large percentage of glycocol on hydrolysis. However, several bacteria (B. paratyphosus, B. coli, B. fecalis-alkaligenes) incapable of liquefying ordinary gelatin mediums, thrive on these glycocol solutions. Investigations using a variety of amino-acids for a considerable number of organisms promise interesting results, and are well under way.

SUMMARY

Four organisms were studied, two of which are strongly proteolytic on ordinary mediums (B. proteus and B. pyocyaneus), one moderately so (staphylococcus), and one (B. typhosus) which does not show the marked activity of the others. On mediums containing ascitic fluid all four destroyed considerable amounts of coagulable protein during the first 36 hours. B. pyocyaneus was the only one which did not do so in the first 12. In the case of the staphylococcus this digestion proceeded at a fairly uniform rate; B. typhosus and B. pyocyaneus apparently did not attack the coagulable protein vigorously until the more easily assimilable substances had been used; B. proteus seems to attack all the nitrogenous materials at once without discrimination. B. proteus and B. typhosus could both assimilate tyrosin and asparagin; B. proteus could make use of glycocol; B. typhosus could not.

DISCUSSION AND CONCLUSIONS

Evidently, the ability of a given cell to assimilate amino-acids does not result directly from their simplicity of structure and solubility in water; otherwise glycocol, which is the simplest and is readily soluble, would be the most easily used. We have been led to believe that the intracellular proteins synthesized by bacteria are specific, in view of various phenomena connected with immunity. It seems reasonable to suppose that this individuality may rest to some extent on the ability to absorb certain substances characteristic of those proteins. Sperry and Rettger found that pure crystallized proteins cannot be attacked by bacteria in the absence of decomposition products; experiments made in this department by Diehl seem to indicate that bacteria will not elaborate a ferment against a certain protein if grown on a medium containing none of the amino-acids found in that protein. The idea of individual requirements in amino-acid by various kinds of bacteria lends color and significance to these observa-

tions. We can think of the free amino-acids present in a culture containing a protein and its split products as both "building stones" in the protein molecule, and as possible "stepping stones" for the bacteria. Then the avidity with which an organism attacks a protein would be in direct proportion to the amount and variety of free amino-acids present which are represented in the structure of the protein molecule and which that particular kind of cell can assimilate. If only a few acids fulfilling both these requirements are found in the medium, the stimulus to proteolysis would not be great, at least until possibly a greater quantity and variety was set free by the first feeble attack. An organism exhibiting what might be called a catholicity of taste in amino-acids would have an increased number of approaches to the protein molecule. Further investigations will show whether or not one is justified in stating that B. proteus, for instance, is such an organism.

In the meanwhile we are certainly justified in concluding that the nature of the particular protein decomposition products present play a very important part in metabolism, as the power to assimilate a given amino-acid is not necessarly common to all bacteria, but is due to factors which may be absent in some varieties.